

CLAIMS

1. A method of producing a chromatographic separation matrix, wherein porous polymeric particles comprised of two layers with different properties are prepared in a two-phase system by
 - 5 (a) providing at least one porous polymeric particle that presents reactive groups on its pore surfaces and on its external surface;
 - (b) washing said particle with a first solvent and draining the particle to enclose a first phase;
 - (c) wetting the enclosing outer layer of the particle by adding a second solvent, which is
10 essentially insoluble in the first solvent, to provide a second phase in the outer layer;
 - (d) reacting the reactive groups in the outer layer by adding a reagent, which is essentially non-reactive in the first solvent; and
 - (e) coupling of chromatographic binding groups to the reactive groups in the inner layer.
2. A method according to claim 1, wherein the reactive groups are carbon-carbon double
15 bonds.
3. A method according to claim 1 or 2, wherein the particle is made from a polymer comprising pendent hydroxy groups, such as agarose.
4. A method according to claim 3, wherein the particle in step (a) is provided by allylation of such hydroxy groups with allyl glycidyl ether (AGE) to provide reactive allyl
20 groups.
5. A method according to any one of claims 1-4, wherein the reagent added in step (d) is an oxidising agent that is reactive in aqueous phases.
6. A method according to any one of claims 1-4, wherein the reagent added in step (d) is an oxidising agent that is reactive in organic phases.
- 25 7. A method according to any one of the preceding claims, wherein the first solvent enclosed in the particle is an organic solvent.
8. A method according to any one of claims 1-6, wherein the first solvent enclosed in the particle is an aqueous solution.
9. A method according to any one of the preceding claims, wherein the aqueous solution
30 comprises an emulgator.

10. A method according to any one of the preceding claims, wherein up to about 30% of the total number of the reactive groups as originally present in the particle are reacted in step (d).
11. A method according to any one of the preceding claims, wherein the coupling according to step (e) is performed by radical activation of allyl groups to allow coupling of binding groups.
12. A method according to any one of the preceding claims, wherein the binding groups of step (e) are ion exchange groups.
13. A method for producing a bifunctional chromatographic separation matrix, which method comprises a method according to any one of the preceding claims, and a further step of modifying the groups in the outer layer and a coupling of chromatographic binding groups to the surface.
14. A porous polymeric particle suitable for use as a chromatographic separation matrix, which is comprised of two layers with different properties, wherein the entire the particle is made from one material and which presents a non-functionalised outer layer.
15. A porous polymeric particle suitable for use as a chromatographic separation matrix, which is comprised of two layers with different properties and which has been produced according to any one of claims 1-13.
16. A process for separating a desired compound from other components in a solution, which is a chromatographic separation method wherein a matrix produced according to any one of claims 1-13 or a matrix comprised of particles according to claim 14 or 15 is used.
17. A process according to claim 16, which is an expanded bed adsorption (EBA) process.
18. A process according to claim 16 or 17, wherein the desired compound is a protein and the solution is a cell lysate.
19. A process according to any one of claims 16-18, wherein the chromatographic separation matrix is an anion exchanger.

20. Use of a chromatographic separation matrix produced by a method according to any one of claims 1-13 or a matrix comprised of particles according to claim 14 or 15 in expanded bed adsorption.